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Original article

Design, synthesis and *in vitro* evaluation of antitubercular and antimicrobial activity of some novel pyranopyrimidines

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ABSTRACT

The clinical significance of pyran and pyrimidine condensed systems and the raise in problem of multidrug resistant bacterial pathogens has directed us to synthesize pyranopyrimidine derivatives via the reactions of the versatile, 2-amino-4-(4-methoxyphenyl)-4*H*-substitutedchromene-3-carbonitrile with the appropriate reagents. The newly synthesized compounds were characterized by IR, ^{1}H NMR, ^{13}C NMR, Mass spectra and Elemental analysis. The compounds were evaluated for their *in vitro* antitubercular activity against *Mycobacterium tuberculosis* $H_{37}Rv$ [ATCC-27294] and antibacterial activity against *Staphylococcus aureus* [ATCC-25923] and *Streptococcus pyogenes* [MTCC-443] as Gram-positive, *Escherichia coli* [ATCC-25922] and *Pseudomonas aeruginosa* [MTCC-441] as Gram-negative bacterial strains and antifungal activity against *Aspergillus niger* [MTCC-282]. Several derivatives exhibited pronounced antitubercular and antimicrobial activities.

1. Introduction

Infectious diseases are influencing the world with their morbidity and mortality out of which tuberculosis is major infectious' diseases caused by *Mycobacterium tuberculosis* [1]. TB (Tuberculosis) is still the single largest infection having a high mortality rate and 0.1–0.3 percent of the population become infected each year in the developed countries. The World Health Organization estimates that 8 million people get TB every year and 3 million people die yearly from TB [2]. The current chemotherapy is based on age-old drugs like Pyrazinamide, Isoniazid and Rifampicin for tuberculosis [3]. The available treatment establishes a multidrug regime lasting a minimum of six months; although there is no guarantee that the complete sterilization of the infection will be obtained. Furthermore, the increase in TB cases caused by MDR and XDR strains, and coinfection with HIV have pointed out the urgent need to develop new antitubercular drugs which will effectively kill MDR strains, less toxic, shortened duration of therapy, rapid mycobactericidal mechanism of action in the intracellular environment.

The benzopyrans have displayed an impressive assay of pharmacological properties like anti HBV, cytotoxic [4], antibacterial [5], antioxidant [6], antigenotoxic [7], ATP sensitive potassium channel openers [8] and antiangiogenic activity [9]. On the other hand pyrimidine scaffold was the base of many bioactive molecules such as antitubercular [10], antibacterial [11], antitumor [12], antiinflammatory [13], antifungal [14] and antileishmanial agent [15]. Consequently, synthetic methodologies for synthesis of novel pyrimidines or pyrimidine fused compounds are of particular interests to organic and medicinal chemists. For example, synthetic methods have been reported for the efficient syntheses of benzopyrano[4,3-*d*]pyrimidine [16] dihydropyrido[2,3-*d*]pyrimidine [17] pyrimido[1,2-*a*]pyrimidine [18] fluorinated 2-amino-pyrimidine-*N*-oxide [19].

As our research is devoted to the synthesis of diverse heterocycles as anti-infective agents, we identified the pyrimidine and pyran (separately) as good antitubercular [20–22] and antimicrobiol agents [23,24]. Keeping this in view we designed new prototypes by combining both pyrimidine and pyran, and synthesized hybrid molecules consisting of pyrimidines along with pyran moiety and investigated for their *in vitro* antitubercular and antimicrobial activities.

2. Results and discussion

2.1. Chemistry

Since the isolation of pyrimidine derivatives, considerable attention has been devoted to their chemistry and biological

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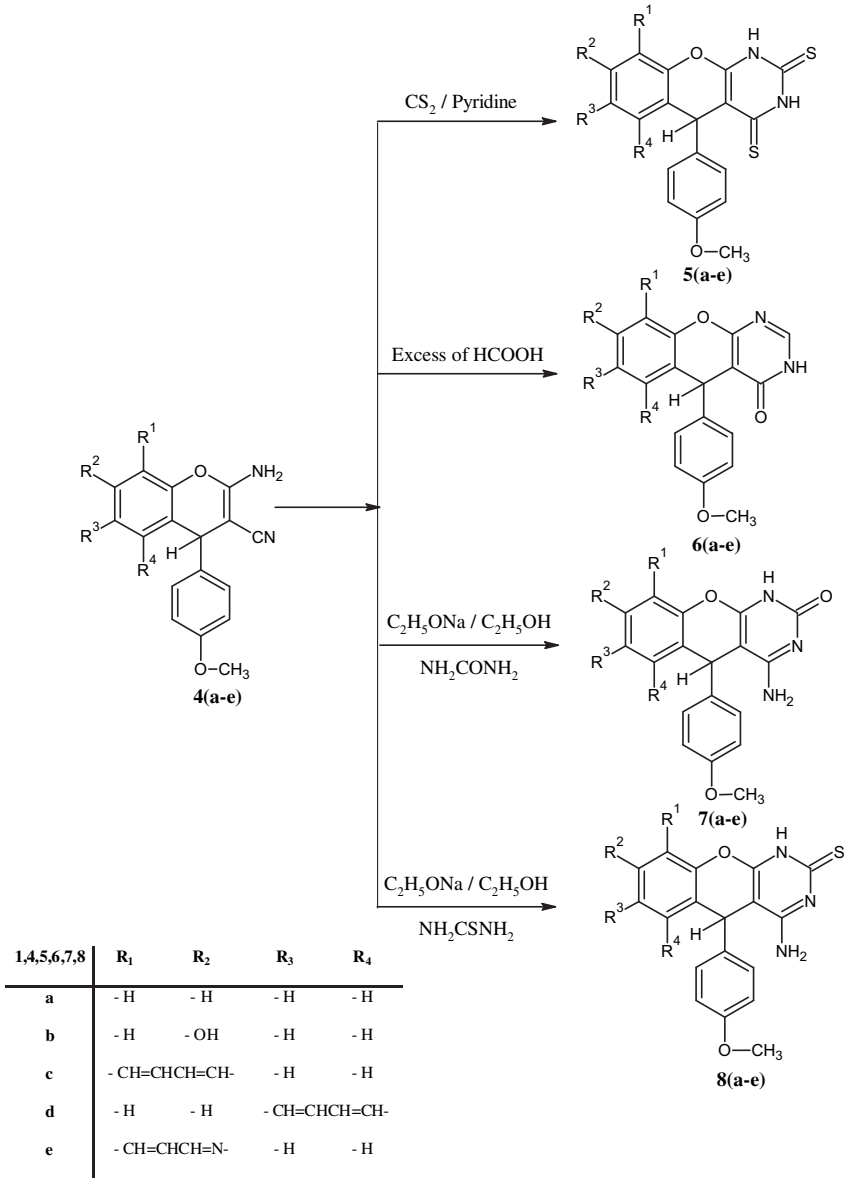
activity. In recent years, there has been increasing interest in the synthesis of pyrimidine derivatives and there are some methods used to synthesize the pyrimidine ring, allowing access to a large number of multifunctionalized pyrimidine derivatives [25–27].

The 2-amino-4-(4-methoxyphenyl)-4*H*-substitutedchromene-3-carbonitrile **4**(**a**–**e**) are easily obtained via one pot reaction of malononitrile, 4-methoxybenzaldehyde and various substituted phenols in presence of cetyltrimethylammoniumchloride [28]. Compounds **4** (**a**–**e**) were used as precursors for the synthesis of new pyrano[4,5-*d*] pyrimidine derivatives. The structure assigned for compound **4**(**a**–**e**) was based on the analytical and spectroscopic data. Reaction of 2-amino-4-(4-methoxyphenyl)-4*H*-substitutedchromene-3-carbonitrile **4**(**a**–**e**) with carbon disulphide in refluxing pyridine affords the corresponding 5-(4-methoxyphenyl)-1,5-dihydro-2H-substitutedchromeno[2,3-*d*]pyrimidine-2,4(3*H*)-dithione (**5a**–**e**). Treatment of different 2-amino-4-(4-methoxyphenyl)-4*H*-substitutedchromene-3-carbonitrile (**4a**–**e**) with formic acid affords the corresponding 5-(4-methoxyphenyl)-3,5-dihydro-4*H*-substitutedchromeno[2,3-*d*]pyrimidine-4-one (**6a**–**e**). Treatment of different 2-amino-4-(4-methoxyphenyl)-4*H*-substitutedchromene-3-carbonitrile (**4a**–**e**) with urea in ethanol and sodium ethoxide affords the corresponding 4-amino-5-(4-methoxyphenyl)-1,5-dihydro-2*H*-substitutedchromeno[2,3-*d*]pyrimidine-2-one **7**(**a**–**e**). An alternative method for the synthesis of compound 4-amino-5-phenyl-1,5-dihydro-2*H*-substitutedchromeno[2,3-*d*]pyrimidine-2-thione (**8a**–**e**) was completed by refluxing 2-amino-4-(4-methoxyphenyl)-4*H*-substitutedchromene-3-carbonitrile **4**(**a**–**e**) with thiourea in ethanol containing sodium ethoxide (Scheme 1).

2.2. Antitubercular activity

All of the synthesized compounds were tested for their antitubercular activity (MIC) *in vitro* by Agar micro dilution method against *M. tuberculosis* $H_{37}Rv$ (ATCC-27294). The results are summarized in Table 1, a standard drug Rifampicin used for comparison. Among the newly synthesized compounds, 7-(4-methoxyphenyl)-7,9-dihydro-8H-pyrimido [5′,4′:5,6] pyrano [3,2-*h*]quinoline-8-one (**6e**) and 8-amino-7-(4-methoxyphenyl)-7,11-dihydro-10H-pyrimido[5′,4′:5,6]pyrano[3,2-*h*]quinoline-10-thione (**8e**) had the highest potency and exhibited inhibition at



1,4,5,6,7,8	R_1	R_2	R_3	R_4
a	- H	- H	- H	- H
b	- H	- OH	- H	- H
c	- CH=CHCH=CH-		- H	- H
d	- H	- H	- CH=CHCH=CH-	
e	- CH=CHCH=N-		- H	- H

Scheme 1.

Table 1
The *in vitro* antitubercular activity of the synthesized compounds against *M. tuberculosis* $H_{37}Rv$.

Compd.	MIC (μg/ml)	Compd.	MIC (μg/ml)	Compd.	MIC (μg/ml)
4a	500	**5e**	125	**7d**	1000
4b	250	**6a**	500	**7e**	250
4c	1000	**6b**	250	**8a**	500
4d	1000	**6c**	125	**8b**	250
4e	250	**6d**	125	**8c**	500
5a	250	**6e**	62.5	**8d**	500
5b	125	**7a**	500	**8e**	62.5
5c	500	**7b**	125	**Rifampicin**	40
5d	500	**7c**	1000		

MIC 62.5 μg/ml which was more potent than the corresponding lead molecule **4**(**a**–**e**) followed by **5b**, **5e**, **6c**, **6d** and **7e** which showed moderate inhibitory activity with MIC 125 μg/ml respectively. The hydroxyl group substituted derivative **6b**, displayed relatively higher inhibitory activity in general. However, replacement of phenyl substitution at chromene with a quinoline improves antitubercular activity. These results clearly showed that **5**,**8**(**a**–**e**) demonstrated more significant antitubercular activity than the corresponding lead molecules. Presence of a hydroxyl and quinoline substituent on chromene caused a remarkable improvement in antitubercular activity.

2.3. Antibacterial activity and antifungal activity

All of the synthesized compounds were tested for their antibacterial and antifungal activity (MIC) *in vitro* by serial dilution technique. Bacterial strains *Staphylococcus aureus* [ATCC-25923] and *Streptococcus pyogenes* [MTCC-443] as Gram-positive, *Escherichia coli* [ATCC-25922] and *Pseudomonas aeruginosa* [MTCC-441] as Gram-negative and *Aspergillus niger* [MTCC-282] as fungal strains were used for the *in vitro* studies. Ampicillin, Ciprofloxacin, Nystatin, and Griseofulvin were use as standard drugs. From the screening results, molecules **5e** and **6e** displayed broad spectrum antimicrobial activity against the both Gram-negative and Gram-positive bacteria compared with ampicillin (Table 2). Compound **5e** showed excellent activity against all bacteria, whereas compound **5b** showed excellent activity against both of the Gram-positive bacteria. Compound **6e** was found significantly active against Gram-negative bacteria *E. coli* compared with ampicillin. Remaining compounds showed good to moderate activity against other bacteria compared with the remaining standard drugs.

3. Conclusion

We report successful synthesis, antitubercular and antimicrobial activity of new pyrimidine and thiopyrimidine derivatives. The assumed structures are confirmed by the IR, ^{1}H NMR, ^{13}C NMR, Mass spectra and Elemental analysis. The antitubercular activity study revealed that all the tested compounds showed good to moderate antitubercular activities against *M. tuberculosis* $H_{37}Rv$. Antimicrobial activity of title compounds showed that presence of hydroxy groups attached to phenyl ring to the chromene ring of the title compounds is responsible for good antimicrobial activity. **5**, **6**, **7**, **8**(**a**–**e**) demonstrated more significant antibacterial, antifungal and antitubercular activity than the corresponding lead molecules. The field is further open for study of these compounds with respect to toxicity, chronic toxicity, pharmacokinetics and clinical studies to establish these molecules as drugs in the market.

4. Experimental

4.1. Chemistry

Melting points were taken in open capillaries and are uncorrected. IR spectra were recorded on a Hitachi 270-50 double beam spectrophotometer in KBr. ^{1}H NMR spectra were recorded on a Varian 300 MHz spectrometer using DMSO-d_6 or $CDCl_3$ as the solvent depending upon the solubility of the synthesized compounds and TMS as the internal standard. Electron impact MS spectra were obtained on a JEOL JMS-HX 100 at 70 eV. Elemental analyses were performed by Perkin Elmer-2400 and were all within ±0.03% of the theoretical values for C, H, and N. The progress of the reaction was monitored by silica gel 60 F_{254} (Merck, 0.25 mm thick) coated TLC plates and column chromatography was performed on silica gel (Merck No. 9385) using suitable mixture of solvents as eluent. The reagent grade chemicals were purchased from the commercial sources and purified by either distillation or recrystallization.

All the synthesized compounds have been checked for their melting points, physical nature, IR, ^{1}H NMR, ^{13}C NMR, Mass spectroscopy and Elemental analysis for individual compounds and the data are summarized as under.

4.2. General preparation of 2-amino-4-(4-methoxyphenyl)-4H-substitutedchromene-3-carbonitrile *4*(*a*–*e*)

A mixture of phenol **1**(**a**–**e**) (2.0 mmol), malononitrile (**2**) (132.0 mg, 2.0 mmol), 4-methoxybenzaldehyde (**3**) (272.0 mg, 2.0 mmol) and catalytic amount of cetyltrimethylammonium-chloride in water (50 ml) was refluxed with continuous stirring for 6 h to give **4**(**a**–**e**).

4.2.1. 2-Amino-4-(4-Methoxyphenyl)-4H-chromene-3-carbonitrile (*4a*)

210 °C (ethanol), White solid, Yield 80%, IR (KBr) ν (cm^{-1}): 2843 (−OCH_3), 3420 (−NH_2), 2200 (−C≡N), 1254 (C−O−C at pyran ring); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 3.81 (s, 3H, −OCH_3), 5.16 (s, 1H, pyran −CH), 7.17 (d, 2H, J = 7.21 Hz, Ar-H), 7.22 (d, 2H, J = 8.01 Hz, Ar-H), 7.02 (d, 2H, J = 7.41 Hz, Ar-H), 6.48–6.65 (t, 2H, Ar-H), 6.80 (s, 2H, −NH_2); *m*/*z*: 278.11 (100.0%), 279.11 (18.6%), 280.11 (2.2%); ^{13}C NMR (300 MHz, $CDCl_3$) δ (ppm): 55.4 (−OCH_3), 15.7 and 131.0 (C=C at pyran ring), 180.4 (C−NH_2), 30.68 (−CH at pyran ring), 80.20 (C−C≡N), 110.22 (−C=N), 119.4, 120.2, 129.3, 128.6, 129.1, 130.2, 132.0, 134.4, 135.4, 136.2 (10 aromatic carbons); Anal. Calcd. for $C_{17}H_{14}N_2O_2$: C, 73.37; H, 5.07; N, 10.07. Found: C, 73.33; H, 5.09; N, 10.05.

4.2.2. 2-Amino-7-hydroxy-4-(4-methoxyphenyl)-4H-chromene-3-carbonitrile (*4b*)

218 °C (ethanol), White solid, Yield 80%, IR (KBr) ν (cm^{-1}): 2840 (−OCH_3), 3390 (−NH_2 stretching), 3220 (−OH), 2200 (−C≡N), 1250 (C−O−C at pyran ring); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 3.84 (s, 3H, −OCH_3), 5.14 (s, 1H pyran), 6.80 (s, 2H, −NH_2), 7.02 (d, 2H, J = 7.39 Hz, Ar-H), 7.42 (d, 1H, J = 8.74 Hz, Ar-H), 7.46 (d, 2H, J = 7.43 Hz, Ar-H), 7.56 (d, 2H, J = 8.34 Hz, Ar-H), 7.66 (s, 1H, −OH); *m*/*z*: 294.10 (100.0%), 295.10 (19.2%), 296.11 (1.6%); ^{13}C NMR (300 MHz, $CDCl_3$) δ (ppm): 55.8 (−OCH_3), 151.25 (C−OH), 145 and 121 (C=C at pyran ring), 177.4 (C−NH_2), 29.68 (−CH at pyran ring), 79.5 (C−C≡N), 111.22 (−C=N), 114.2, 114.5, 119.3, 128.6, 129.1, 130.2, 132.0, 134.4, 135.4 (9 aromatic carbons); Anal. Calcd. for $C_{17}H_{14}N_2O_3$: C, 69.38; H, 4.79; N, 9.52. Found: C, 69.38; H, 4.79; N, 9.52.

4.2.3. 2-Amino-4-(4-methoxyphenyl)-4H-benzo[h]chromene-3-carbonitrile (*4c*)

215 °C (ethanol), White solid, Yield 90%, IR (KBr) ν (cm^{-1}): 2845 (−OCH_3), 3410 (−NH_2), 2197 (−C≡N), 1254 (C−O−C at pyran ring);

Table 2
The *in vitro* antimicrobial activity (MIC, μg/ml) of the synthesized compounds.

Compd.	The minimum inhibitory concentration (MIC)				
	Gram - ive		Gram + ive		Antifungal
	Escherichia coli [ATCC-25922]	*Pseudomonas aeruginosa* [MTCC − 441]	*Streptococcus pyogenes* [MTCC − 443]	*Staphylococcus aureus* [ATCC-25923]	*Aspergillus niger* [MTCC − 282]
4a	500	500	250	250	1000
4b	250	250	125	125	500
4c	500	1000	500	500	500
4d	150	500	250	250	1000
4e	250	250	125	125	500
5a	250	250	125	125	500
5b	125	250	62.5	62.5	500
5c	500	500	250	250	125
5d	250	250	250	125	1000
5e	125	125	62.5	62.5	250
6a	500	500	250	250	1000
6b	500	500	250	250	500
6c	250	250	125	125	1000
6d	250	125	250	125	1000
6e	62.5	125	125	125	125
7a	250	250	125	125	250
7b	250	125	250	125	250
7c	500	250	250	250	500
7d	250	500	125	125	500
7e	125	125	125	62.5	250
8a	250	250	125	125	500
8b	250	250	62.5	62.5	250
8c	500	250	500	500	1000
8d	500	500	500	250	1000
8e	125	125	62.5	62.5	500
Ciprofloxacin	25	25	50	50	–
Ampicillin	100	100	250	100	–
Nystatin	–	–	–	–	100
Greseofulvin	–	–	–	–	100

^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 3.85 (s, 3H, $-OCH_3$), 5.13 (s, 1H, pyran), 6.88 (s, 2H, $-NH_2$), 7.25 (d, 2H, $J = 7.21$ Hz, Ar-H), 7.32 (d, 2H, $J = 8.02$ Hz, Ar-H), 7.46 (d, 2H, $J = 7.41$ Hz, Ar-H), 7.60 (d, 2H, $J = 8.25$ Hz, Ar-H), 7.66 (t, 2H, Ar-H); *m/z*: 328.12 (100.0%), 329.12 (23.5%), 330.13 (2.9%); ^{13}C NMR (300 MHz, $CDCl_3$) δ (ppm): 55.6 ($-OCH_3$), 151.0 and 122.0 (C═C at pyran ring), 178.4 (C—NH_2), 30.1 (−CH at pyran ring)00, 79.4 (C—C≡N), 109.20 (−C═N), 118.0, 119.5, 121.2, 123.5, 125.9, 127.3, 128.6, 129.1, 130.2, 132.0, 134.4, 135.4, 136.2, 140.2 (14 aromatic carbons); Anal. Calcd. for $C_{21}H_{16}N_2O_2$: C, 76.81; H, 4.91; N, 8.53. Found: C, 76.82; H, 4.95; N, 8.50.

4.2.4. 3-Amino-1-(4-Methoxyphenyl)-1H-benzo[f]chromene-2-carbonitrile (**4d**)

278 °C (ethanol), Yellow solid, Yield 74%, IR (KBr) ν (cm^{-1}): 2841 ($-OCH_3$), 3420 ($-NH_2$ stretching), 2210 (−C≡N) 1257 (C—O—C at pyran ring); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 3.83 (s, 3H, $-OCH_3$), 5.31 (s, 1H, pyran −CH), 7.20 (d, 2H, $J = 7.27$ Hz, Ar-H), 7.36 (d, 2H, $J = 8.0$ Hz, Ar-H), 7.44 (d, 2H, $J = 7.4$ Hz, Ar-H), 7.62 (d, 2H, $J = 8.2$ Hz, Ar-H), 7.80 (t, 2H, Ar-H), 6.9 (s, 2H, $-NH_2$); *m/z*: 328.12 (100.0%), 329.12 (23.5%), 330.13 (2.9%); ^{13}C NMR (300 MHz, $CDCl_3$) δ (ppm): 55.6 (OCH_3), 151.0 and 122.0 (C═C at pyran ring), 178.4 (C—NH_2), 30.1 (−CH at pyran ring), 79.4 (C—C≡N), 109.20 (−C═N), 118.0, 119.5, 121.2, 123.5, 125.9, 127.3, 128.6, 129.1, 130.2, 132.0, 134.4, 135.4, 136.2, 140.2 (14 aromatic carbons); Anal. Calcd. for $C_{21}H_{16}N_2O_2$: C, 76.81; H, 4.91; N, 8.53. Found: C, 76.82; H, 4.95; N, 8.51.

4.2.5. 2-Amino-4-(4-Methoxyphenyl)-4H-pyrano[3,2-h]quinoline-3-carbonitrile (**4e**)

270 °C (ethanol), Yellow solid, Yield 85%, IR (KBr) ν (cm^{-1}): 2846 ($-OCH_3$), 3390 ($-NH_2$ stretching), 2200 (−C≡N), 1253 (C—O—C at pyran ring); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 3.81 (s, 3H, $-OCH_3$), 5.18 (s, 1H pyran −CH), 6.9 (s, 2H, $-NH_2$), 7.34 (d, 2H, $J = 7.20$ Hz, Ar-H), 7.39 (d, 2H, $J = 8.08$ Hz, Ar-H), 7.48 (d, 2H, $J = 7.49$ Hz, Ar-H), 7.60 (t, 1H, Ar-H), 7.85 (t, 2H, Ar-H), 7.56 (d, 2H, $J = 7.64$ Hz, Ar-H at pyridine ring); *m/z*: 329.12 (100.0%), 330.12 (21.9%), 331.12 (2.9%), 330.11 (1.1%); ^{13}C NMR (300 MHz, $CDCl_3$) δ (ppm): 55.8 (OCH_3), 151.0 and 122.0 (C═C at pyran ring), 178.4 (C—NH_2), 79.4 (C—C≡N), 109.20 (−C═N), 45.0 (−CH at pyran ring), 120.1, 126.2, 133.9, 135.0 (carbons at pyridine ring), 150.0 (−C═N at pyridine ring), 129.6, 128.2, 129.8, 130.9, 132.6, 134.9, 135.4, 136.2 (aromatic carbons); Anal. Calcd. for $C_{20}H_{15}N_3O_2$: C, 72.94; H, 4.52; N, 12.76. Found: C, 72.93; H, 4.54; N, 12.75.

4.3. General preparation of 5-(4-methoxyphenyl)-1,5-dihydro-2H-substitutedchromeno[2,3-d]pyrimidine-2,4(3H)-dithione **5**(**a**–**e**)

A mixture of compound **4**(**a**–**e**) (2.0 mmol) and carbon disulphide (152.0 mg, 2.0 mmol) in pyridine (10 ml) were refluxed on water bath for 6 h (Monitored by TLC). After completion of reaction, the reaction mixture was cooled at room temperature then poured in to ice cold water (50 ml) and neutralized with hydrochloric acid (1:1). The separate product was filtered off, washed and recrystalized from ethanol.

4.3.1. 5-(4-Methoxyphenyl)-1,5-dihydro-2H-chromeno[2,3-d] pyrimidine-2,4(3H)-dithione (**5a**)

162–163 °C (ethanol), Yellow solid, Yield 74%, IR (KBr) ν (cm^{-1}): 3337, 3212 (−NH stretching), 2843 ($-OCH_3$), 1508, 1646, 1622 (−NH bending), 1442, 1254 (C—O—C at pyran ring), 1307 (>C═S pyrimidine dithione ring); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 3.83 (s, 3H, $-OCH_3$), 4.71 (s, 1H, pyran −CH), 8.56 (s, 1H, −NH), 7.03 (d, 2H, $J = 7.11$ Hz, Ar-H), 7.12 (d, 2H, $J = 8.10$ Hz, Ar-H), 7.22 (d, 2H, $J = 7.43$ Hz, Ar-H), 6.48–6.75 (t, 2H, Ar-H); *m/z*: 354.05 (100.0%), 355.05 (21.9%), 356.05 (9.9%), 356.06 (1.8%), 357.05 (1.8%); ^{13}C NMR (300 MHz, $CDCl_3$) δ (ppm): 150.7, 166.0 (C—O—C at pyran ring), 45.0 (−CH at pyran ring), 55.8 ($-OCH_3$), 177.0, 194.9 (>C═S at

pyrimidine dithione ring), 118.0, 119.4, 120.2, 129.3, 128.6, 129.1, 130.2, 132.0, 134.4, 135.4, 136.2 (aromatic carbons); Anal. Calcd. for $C_{18}H_{14}N_2O_2S_2$ (354.45): C, 60.99; H, 3.98; N, 7.90. Found: C, 61.02; H, 3.96; N, 7.93%.

*4.3.2. 8-Hydroxy-5-(4-methoxyphenyl)-1,5-dihydro-2H-chromeno [2,3-d]pyrimidine-2,4(3H)-dithione (**5b**)*

181–183 °C (ethanol), Yellow solid, Yield 81%, IR (KBr) ν (cm^{-1}): 3428, 3299 (−NH stretching), 2842 (−OCH_3), 1510, 1637, 1625, (−NH bending), 3200 (−OH), 1448, 1246 (C−O−C at pyran ring), 1315 (>C=S at pyrimidine dithione ring); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 3.83 (s, 3H, −OCH_3), 4.74 (s, 1H, pyran −CH), 8.58 (s, 1H, −NH), 8.98 (s, 1H, −OH), 6.58 (d, 2H, J = 7.45 Hz, Ar-H), 7.32 (d, 1H, J = 8.89 Hz, Ar-H), 7.47 (d, 2H, J = 7.42 Hz, Ar-H), 7.85 (d, 2H, J = 8.51 Hz, Ar-H); m/z: 370.04 (100.0%), 371.05 (19.7%), 372.04 (9.2%), 372.05 (2.8%), 371.04 (2.3%), 373.04 (1.9%); ^{13}C NMR (300 MHz, $CDCl_3$) δ (ppm): 55.83 (−OCH_3), 151.1, 168.0 (C−O−C at pyran ring), 160.0 (C−OH), 43.1 (−CH at pyran ring), 177.5 and 194.7 (>C=S at pyrimidine dithione ring), 119.4, 120.2, 129.3, 128.6, 129.1, 130.2, 132.0, 134.4, 135.4, 136.2 (aromatic carbons); Anal. Calcd. for $C_{18}H_{14}N_2O_3S_2$ (370.45): C, 58.36; H, 3.82; N, 7.56. Found: C, 58.37; H, 3.80; N, 7.53%.

*4.3.3. 7-(4-Methoxyphenyl)-7H-benzo[7, 8]chromeno[2,3-d] pyrimidine-8,10(9H,11H)-dithione (**5c**)*

165–167 °C (ethanol), Yellow solid, Yield 71%, IR (KBr) ν (cm^{-1}): 3455, 3343 (−NH stretching), 2839 (−OCH_3), 1513, 1648, 1620 (−NH bending), 1434, 1249 (C−O−C at pyran ring), 1303 (>C=S at pyrimidine dithione ring); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 3.76 (s, 3H, −OCH_3), 4.77 (s, 1H, pyran −CH), 8.54 (s, 1H, −NH), 7.01 (d, 2H, J = 7.18 Hz, Ar-H), 7.21 (d, 2H, J = 8.11 Hz, Ar-H), 7.60 (d, 2H, J = 7.46 Hz, Ar-H), 7.77 (d, 2H, J = 8.29 Hz, Ar-H), 7.89 (t, 2H, Ar-H); m/z: 404.07 (100.0%), 405.07 (24.1%), 406.06 (9.1%), 406.07 (3.7%), 405.06 (2.3%), 407.06 (2.3%); ^{13}C NMR (300 MHz, $CDCl_3$) δ (ppm): 55.76 (−OCH_3), 152.1, 170.0 (C−O−C at pyran ring) 44.9 (−CH at pyran ring), 177.5, 194.7 (>C=S at pyrimidine dithione ring), 119.4, 120.2, 123.9, 155, 129.3, 128.6, 129.1, 130.2, 132.0, 134.4, 135.4, 136.2 (aromatic carbons); Anal. Calcd. for $C_{22}H_{16}N_2O_2S_2$ (404.50): C, 65.32; H, 3.99; N, 6.93. Found: C, 65.29; H, 4.03; N, 6.95%.

*4.3.4. 12-(4-Methoxyphenyl)-8,12-dihydro-9H-benzo[5,6] chromeno[2,3-d]pyrimidine-9,11(10H)-dithione (**5d**)*

175–177 °C (ethanol), Yellow solid, Yield 67%, IR (KBr) ν (cm^{-1}): 3432, 3339 (−NH stretching), 2844 (−OCH_3), 1503, 1639, 1627 (−NH bending), 1436, 1266 (C−O−C at pyran ring), 1292 (>C=S at pyrimidine dithione ring); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 3.84 (s, 3H, −OCH_3), 4.74 (s, 1H, pyran −CH), 8.61 (s, 1H, −NH), 7.24 (d, 2H, J = 7.28 Hz, Ar-H), 7.46 (d, 2H, J = 8.19 Hz, Ar-H), 7.64 (d, 2H, J = 7.38 Hz, Ar-H), 7.72 (d, 2H, J = 8.29 Hz, Ar-H), 8.20 (t, 2H, Ar-H); m/z: 404.07 (100.0%), 405.07 (24.1%), 406.06 (9.1%), 406.07 (3.7%), 405.06 (2.3%), 407.06 (2.3%); ^{13}C NMR (300 MHz, $CDCl_3$) δ (ppm): 55.84 (−OCH_3), 152.1, 170.0 (C−O−C at pyran ring), 44.9 (−CH at pyran ring), 177.5, 194.7 (>C=S at pyrimidine dithione ring), 119.6, 120.3, 123.9, 125.0, 129.1, 128.4, 129.4, 130.0, 132.2, 134.7, 135.5, 136.4 (aromatic carbons); Anal. Calcd. for $C_{22}H_{16}N_2O_2S_2$ (404.50): C, 65.32; H, 3.99; N, 6.93. Found: C, 65.30; H, 4.02; N, 6.96%.

*4.3.5. 7-(4-Methoxyphenyl)-7H-pyrimido[5′,4′:5,6]pyrano[3,2-h] quinoline-8,10(9H,11H)-dithione (**5e**)*

187–189 °C (ethanol), Brown solid, Yield 59%, IR (KBr) ν (cm^{-1}): 3455, 3329 (−NH stretching), 2845 (−OCH_3), 1519, 1640, 1631 (−NH Bending), 1447, 1249 (C−O−C at pyran ring), 1313 (>C=S at pyrimidine dithione ring); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 3.83 (s, 3H, OCH_3), 4.78 (s, 1H pyran −CH), 8.26 (s, 1H, −NH), 7.30–7.56 (m, 3H, Ar-H at pyridine ring), 7.34 (d, 2H, J = 7.21 Hz, Ar-H), 7.41 (d, 2H, J = 8.09 Hz, Ar-H), 7.49 (d, 2H, J = 7.33 Hz, Ar-H), 7.65 (t, 1H, Ar-H), 7.68 (t, 2H, Ar-H), 8.27 (d, 2H, J = 7.69 Hz, Ar-H at pyridine ring); m/z: 405.06 (100.0%), 406.06 (25.5%), 407.06 (10.1%), 407.07 (2.5%), 408.06 (2.2%). ^{13}C NMR (300 MHz, $CDCl_3$) δ (ppm): 55.83 (−OCH_3), 151.1, 169.0 (C−O−C at pyran ring), 44.0 (−CH at pyran ring), 178.2, 194.7 (>C=S at pyrimidine dithione ring), 120.1, 126.2, 133.9, 135.0 (carbons at pyridine ring), 150.0 (−C=N at pyridine ring), 129.6, 128.2, 129.8, 130.9, 132.6, 134.9, 135.4, 136.2 (aromatic carbons); Anal. Calcd. for $C_{21}H_{15}N_3O_2S_2$ (405.49): C, 62.20; H, 3.73; N, 10.36. Found: C, 62.22; H, 3.71; N, 10.39%.

*4.4. General preparation of 5-(4-methoxyphenyl)-3,5-dihydro-4H-substitutedchromeno[2,3-d]pyrimidine-4-one **6**(**a**–**e**)*

A mixture of compound **4**(**a**–**e**) (2.0 mmol) and excess of formic acid were refluxed on sand bath for 12 h (Monitored by TLC). After completion of the reaction, solvent was distilled off under reduced pressure and the solid thus obtained was purified by recrystallization from absolute ethanol to give compounds **6**(**a**–**e**).

*4.4.1. 5-[4-Methoxyphenyl]-3,5-dihydro-4H-chromeno[2,3-d] pyrimidine-4-one (**6a**)*

154–156 °C (ethanol), Cream solid, Yield 71%, IR (KBr) ν (cm^{-1}): 3372 (−NH stretching), 1690 (>C=O), 2843 (−OCH_3), 1610 (−C=N at pyrimidine ring), 1515 (−NH bending), 1441, 1256 (C−O−C at pyran ring); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 3.81 (−OCH_3), 4.74 (s, 1H, pyran −CH), 7.92 (d, 1H, −NH at pyrimidine ring), 7.64 (d, 1H, −CH at pyrimidine ring), 7.12 (d, 2H, J = 7.23 Hz, Ar-H), 7.20 (d, 2H, J = 8.16 Hz, Ar-H), 7.37 (d, 2H, J = 7.45 Hz, Ar-H), 6.83–6.90 (t, 2H, Ar-H); m/z: 306.10 (100.0%), 307.10 (20.3%), 308.11 (1.8%); ^{13}C NMR (300 MHz, $CDCl_3$) δ (ppm): 55.43 (−OCH_3), 155.5, 152.9 (C−O−C at pyran ring), 39.3 (−CH at pyran ring), 150.2 (−C=N at pyrimidine ring), 162.1.0 (>C=O), 106.1, 108.5, 110.5, 113.6, 120.2, 123.3, 125.9, 128.4, 129.2, 130.3 (aromatic carbons); Anal. Calcd. for $C_{18}H_{14}N_2O_3$ (306.31): C, 70.58; H, 4.61; N, 9.15. Found: C, 70.60; H, 4.59; N, 9.16%.

*4.4.2. 8-Hydroxy-5-(4-methoxyphenyl)-3,5-dihydro-4H-chromeno [2,3-d]pyrimidine-4-one (**6b**)*

165–168 °C (ethanol), White solid, Yield 55%, IR (KBr) ν (cm^{-1}): 3381 (−NH stretching), 1697 (>C=O stretching), 2840 (−OCH_3), 1616 (−C=N at pyrimidine ring), 1511 (−NH bending), 1457, 1239 (C−O−C at pyran ring), 3200 (−OH); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 3.84 (−OCH_3), 4.81 (s, 1H, pyran −CH), 8.12 (d, 1H, −NH at pyrimidine ring), 7.50 (d, 1H, −CH at pyrimidine ring), 9.0 (s, 1H, −OH), 6.77 (d, 2H, J = 7.51 Hz, Ar-H), 7.19 (d, 1H, J = 8.77 Hz, Ar-H), 7.24 (d, 2H, J = 7.33 Hz, Ar-H), 7.30 (d, 2H, J = 8.39 Hz, Ar-H); m/z: 322.10 (100.0%) 323.10(19.8%), 324.10 (2.8%); ^{13}C NMR (300 MHz, $CDCl_3$) δ (ppm): 55.42 (−OCH_3) 156.5, 154.5 (C−O−C at pyran ring), 40.0 (−CH at pyran ring), 150.6 (−C=N at pyrimidine ring), 161.3 (>C=O), 162.6 (C−OH), 101.1, 108.5, 111.5, 123.6, 123.2, 125.3, 125.9, 128.4, 129.2, 130.3, 131.0 (aromatic carbons); Anal. Calcd. for $C_{18}H_{14}N_2O_4$ (322.31): C, 67.07; H, 4.38; N, 8.69. Found: C, 67.09; H, 4.35; N, 8.72%.

*4.4.3. 7-(4-Methoxyphenyl)-7,9-dihydro-8H-benzo[7,8]chromeno [2,3-d]pyrimidine-8-one (**6c**)*

153–156 °C (ethanol), Brown solid, Yield 64%, IR (KBr) ν (cm^{-1}): 3364 (−NH stretching), 1705 (>C=O), 2848 (−OCH_3), 1602 (−C=N at pyrimidine ring), 1523 (−NH bending), 1450, 1266 (C−O−C at pyran ring); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): δ 3.85 (−OCH_3), 4.80 (s, 1H, pyran −CH), 8.10 (d, 1H, −NH at pyrimidine ring), 7.55 (d, 1H, −CH at pyrimidine ring), 6.50 (d, 2H, J = 7.22 Hz, Ar-H), 6.87 (d, 2H, J = 7.99 Hz, Ar-H), 6.99 (d, 2H, J = 7.53 Hz, Ar-H), 7.14 (d, 2H, J = 8.25 Hz, Ar-H), 7.25 (t, 2H, Ar-H); m/z: 356.12 (100.0%), 357.12

(24.1%), 358.12 (3.5%); ^{13}C NMR (300 MHz, $CDCl_3$) δ (ppm): 55.44 (−OCH_3), 144.0–151.0 (C–O–C at pyran ring), 37.9 (−CH at pyran ring), 151.8 (−C=N at pyrimidine ring), 161.2 (>C=O), 118.2, 120.7, 121.8, 122.6, 123.2, 125.8, 126.9, 127.4, 128.6, 129.9, 130.4, 130.2 (aromatic carbons); Anal. Calcd. for $C_{22}H_{16}N_2O_3$ (356.37): C, 74.15; H, 4.53; N, 7.86. Found: C, 74.18; H, 4.55; N, 7.83%.

4.4.4. 12-(4-Methoxyphenyl)-10,12-dihydro-11H-benzo[5,6] chromeno[2,3-d]pyrimidine-11-one (**6d**)

155–157 °C (ethanol), Yellow solid, Yield 68%, IR (KBr) ν (cm^{-1}): 3389 (−NH stretching), 2840 (−OCH_3), 1725 (>C=O), 1618 (−C=N at pyrimidine ring), 1522 (−NH bending), 1460, 1221 (C–O–C at pyran ring); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 3.80 (−OCH_3), 4.85 (s, 1H, pyran −CH), 8.15 (d, 1H, −NH at pyrimidine ring), 7.52 (d, 1H, −CH at pyrimidine ring), 6.77 (d, 2H, J = 7.21 Hz, Ar-H), 7.12 (d, 2H, J = 8.10 Hz, Ar-H), 7.44 (d, 2H, J = 7.47 Hz, Ar-H), 7.45 (d, 2H, J = 8.26 Hz, Ar-H), 7.65 (t, 2H, Ar-H); m/z: 356.12 (100.0%), 357.12 (24.1%), 358.12 (3.5%); ^{13}C NMR (300 MHz, $CDCl_3$) δ (ppm): 55.84 (−OCH_3), 145.0, 151.0 (C–O–C at pyran ring) 37.6 (−CH at pyran ring), 151.2 (−C=N at pyrimidine ring), 161.4 (>C=O), 118.2, 120.7, 121.8, 122.6, 123.2, 125.8, 126.9, 127.4, 128.6, 129.9, 130.4, 130.2 (aromatic carbons). Anal. Calcd. for $C_{21}H_{14}N_2O_2$ (356.37): C, 74.15; H, 4.53; N, 7.86. Found: C, 74.16; H, 4.56; N, 7.84%.

4.4.5. 7-(4-Methoxyphenyl)-7,9-dihydro-8H-pyrimido[5′,4′:5,6] pyrano[3,2-h]quinoline-8-one (**6e**)

170–172 °C (ethanol), Yellow solid, Yield 63%, IR (KBr) ν (cm^{-1}): 3376 (−NH stretching), 1730 (>C=O), 2842 (−OCH_3), 1601 (−C=N at pyrimidine ring), 1504 (−NH bending), 1448, 1247 (C–O–C at pryan ring); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 3.84 (−OCH_3), 4.92 (s, 1H, pyran −CH), 8.10 (d, 1H, −NH at pyrimidine ring), 7.56 (d, 2H, J = 7.55 Hz, Ar-H at pyridine ring), 7.34–7.49 (m, 3H, Ar-H at pyridine ring), 6.83 (d, 2H, J = 7.29 Hz, Ar-H), 7.21 (d, 2H, J = 8.15 Hz, Ar-H), 7.28 (d, 2H, J = 7.41 Hz, Ar-H), 7.47 (t, 1H, Ar-H), 7.63 (t, 2H, Ar-H); m/z: 357.11 (100.0%), 358.11 (23.8%), 359.12 (3.1%); ^{13}C NMR (300 MHz, $CDCl_3$) δ (ppm): 55.84 (−OCH_3), 162.2, 151.4 (C–O–C at pyran ring), 39.3 (−CH at pyran ring), 150.0 (−C=N at pyrimidine ring), 162.6 (>C=O), 120.1, 127.2, 135.2, 137.6 (carbons at pyridine ring), 150.7 (−C=N at pyridine ring), 128.6, 131.2, 131.8, 132.9, 133.6, 134.9, 135.4, 136.2 (aromatic carbons); Anal. Calcd. for $C_{21}H_{15}N_3O_3$ (357.36): C, 70.58; H, 4.23; N, 11.76. Found: C, 70.61; H, 4.25; N, 11.74%.

4.5. General preparation of 4-amino-5-(4-methoxyphenyl)-1,5-dihydro-2H-substituted chromeno[2,3-d]pyrimidine-2-one **7**(**a**–**e**)

A mixture of compound **4**(**a**–**e**) (2.0 mmol) and urea (120.0 mg, 2.0 mmol) with catalytic amount of sodium ethoxide in ethanol (15 ml) was refluxed on water bath for 6–7 h (Monitored by TLC). After completion of reaction, the reaction mixture was poured in crushed ice (50 g) and neutralized by the diluted hydrochloric acid (1:1). The separated product was collected by the filtration and washed with water. The crude product was purified by crystallization from absolute ethanol **7**(**a**–**e**).

4.5.1. 4-Amino-5-(4-methoxyphenyl)-1,5-dihydro-2H-chromeno [2,3-d]pyrimidine-2-one (**7a**)

168–172 °C (ethanol), Brown solid, Yield 58%, IR (KBr) ν (cm^{-1}): 3446 (−NH stretching at NH_2), 1605, 1509 (−NH bending at −NH_2), 3301 (−NH stretching at pyrimidine ring), 2844 (−OCH_3), 1653 (>C=O), 1579 (−C=N at pyrimidine ring), 1454, 1400 (C–N at pyrimidine ring), 1448, 1260 (C–O–C at pyran ring); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 3.84 (−OCH_3), 4.84 (s, 1H, pyran −CH), 8.15 (s, 2H, −NH_2), 8.21 (s, 1H, −NH at pyrimidine ring), 6.78 (d, 2H, J = 7.17 Hz, Ar-H), 7.26 (d, 2H, J = 8.13 Hz, Ar-H), 7.45 (d, 2H, J = 7.52 Hz, Ar-H), 6.89–7.10 (t, 2H, Ar-H); m/z: 321.11 (100.0%), 322.11 (20.6%), 323.12 (2.5%); ^{13}C NMR (300 MHz, $CDCl_3$) δ (ppm): 55.44 (−OCH_3) 150.3, 148.9 (C–O–C at pyran ring), 33.8 (−CH at pyran ring), 163.3 (C–NH_2), 162.5 (>C=O), 122.5, 122.5, 123.6, 124.2, 125.3, 127.9, 128.4, 129.2, 130.3, 131.0 (aromatic carbons); Anal. Calcd. for $C_{18}H_{15}N_3O_3$ (321.33): C, 67.28; H, 4.71; N, 13.08. Calcd: C, 67.25; H, 4.74; N, 13.05%.

4.5.2. 4-Amino-8-hydroxy-5-(4-methoxyphenyl)-1,5-dihydro-2H-chromeno[2,3-d]pyrimidine-2-one (**7b**)

170–173 °C (ethanol), Brown solid, Yield 75%, IR (KBr) ν (cm^{-1}): 3450 (−NH stretching at NH_2), 1613, 1510 (−NH bending at −NH_2), 3306 (−NH stretching at pyrimidine ring), 3215 (−OH), 2849 (−OCH_3), 1667 (>C=O), 1580 (−C=N at pyrimidine ring), 1456, 1407 (C–N pyrimidine ring), 1437, 1265 (C–O–C at pyran ring); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 3.79 (−OCH_3), 4.76 (s, 1H, pyran −CH), 8.0 (s, 2H, −NH_2), 8.12 (s, 1H, −NH at pyrimidine ring), 9.09 (s, 1H, −OH), 6.85 (d, 2H, J = 7.44 Hz, Ar-H), 7.11 (d, 1H, J = 8.77 Hz, Ar-H), 7.26 (d, 2H, J = 7.48 Hz, Ar-H), 7.30 (d, 2H, J = 8.49 Hz, Ar-H); m/z: 337.11 (100.0%), 338.11 (19.8%), 339.11 (2.9%), 338.10 (1.1%); ^{13}C NMR (300 MHz, $CDCl_3$) δ (ppm): 55.79 (−OCH_3), 154.5, 136.2 (C–O–C at pyran ring), 34.4 (−CH at pyran ring), 164.6 (C–NH_2), 161.3 (>C=O), 162.8 (C–OH), 100.2, 110.5, 111.5, 123.6, 123.2, 125.3, 125.9, 128.4, 129.2, 130.3, 131.0 (aromatic carbons); Anal. Calcd. for $C_{18}H_{15}N_3O_4$ (355.34): C, 60.09; H, 4.48; N, 12.46. Found: C, 60.05; H, 4.46; N, 12.49%.

4.5.3. 8-Amino-7-(4-methoxyphenyl)-7,11-dihydro-10H-benzo[7,8] chromeno[2,3-d]pyrimidine-10-one (**7c**)

172–175 °C (ethanol), Green solid, Yield 68%, IR (KBr) ν (cm^{-1}): 3448 (−NH stretching at NH_2), 1604, 1512 (−NH bending at −NH_2), 3311 (−NH stretching at pyrimidine ring), 2839 (−OCH_3), 1657 (>C=O stretching), 1575 (−C=N at pyrimidine ring), 1444, 1416 (C–N at pyrimidine ring), 1264 (C–O–C at pyran ring); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 3.79 (−OCH_3), 4.75 (s, 1H, pyran −CH), 8.05 (s, 2H, −NH_2), 8.16 (s, 1H, −NH at pyrimidine ring), 7.03 (d, 2H, J = 7.28 Hz, Ar-H), 7.18 (d, 2H, J = 8.12 Hz, Ar-H), 7.44 (d, 2H, J = 7.55 Hz, Ar-H), 7.69 (d, 2H, J = 8.33 Hz, Ar-H), 7.89 (t, 2H, Ar-H); m/z: 371.13 (100.0%), 372.13 (24.1%), 373.13 (3.6%), 372.12 (1.1%); ^{13}C NMR (300 MHz, $CDCl_3$) δ (ppm): 55.79 (−OCH_3), 136.0, 151.0 (C–O–C at pyran ring), 32.7 (−CH at pyran ring), 164.6 (C–NH_2), 148.4 (>C=O), 123.2, 122.7, 123.8, 123.6, 124.2, 125.8, 126.9, 127.4, 128.6, 129.9, 130.4, 130.2 (aromatic carbons); Anal. Calcd. for $C_{22}H_{17}N_3O_3$ (371.38): C, 71.15; H, 4.61; N, 11.31. Found: C, 71.14; H, 4.63; N, 11.28%.

4.5.4. 11-Amino-12-(4-methoxyphenyl)-8,12-dihydro-9H-benzo [5,6]chromeno[2,3-d]pyrimidine-9-one (**7d**)

190–192 °C (ethanol), Brown solid, Yield 74%, IR (KBr) ν (cm^{-1}): 3446 (−NH stretching at NH_2), 1615, 1505 (−NH bending at −NH_2), 3322 (−NH stretching at pyrimidine ring), 2850 (−OCH_3), 1643 (>C=O), 1579 (−C=N at pyrimidine ring), 1452, 1419 (C–N at pyrimidine ring), 1267 (C–O–C at pyran ring); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 3.85 (−OCH_3), 4.78 (s, 1H, pyran −CH), 7.95 (s, 2H, −NH_2), 8.05 (s, 1H, −NH at pyrimidine ring), 6.89 (d, 2H, J = 7.10 Hz, Ar-H), 7.14 (d, 2H, J = 8.11 Hz, Ar-H), 7.23 (d, 2H, J = 7.39 Hz, Ar-H), 7.30 (d, 2H, J = 8.29 Hz, Ar-H), 7.35 (t, 2H, Ar-H); m/z: 371.13 (100.0%), 372.13 (24.1%), 373.13 (3.6%), 372.12 (1.1%); ^{13}C NMR (300 MHz, $CDCl_3$) δ (ppm): 55.79 (−OCH_3), 136.0, 151.0 (C–O–C at pyran ring), 32.7 (−CH at pyran ring), 164.6 (C–NH_2), 158.4 (>C=O), 123.2, 122.7, 123.8, 123.6, 124.2, 125.8, 126.9, 127.4, 128.6, 129.9, 130.4, 130.2 (aromatic carbons); Anal. Calcd. for $C_{22}H_{17}N_3O_3$ (371.38): C, 71.15; H, 4.61; N, 11.31. Found: C, 71.17; H, 4.64; N, 11.30%.

4.5.5. 8-Amino-7-(4-methoxyphenyl)-7,11-dihydro-10H-pyrimido [5′,4′:5,6]pyrano[3,2-h]quinoline-10-one (**7e**)

168–170 °C (ethanol), Yellow solid, Yield 78%, IR (KBr) ν (cm^{-1}): 3446 (−NH stretching at NH_2), 1614, 1508 (−NH bending at −NH_2),

3313 (−NH stretching at pyrimidine ring), 2835 (−OCH_3), 1649 (>C=O stretching), 1580 (−C=N at pyrimidine ring), 1455, 1405 (C−N at pyrimidine ring), 1250 (C−O−C at pyran ring); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 3.86 (−OCH_3), 4.84 (s, 1H, pyran −CH), 8.24 (s, 2H, −NH_2), 8.11 (s, 1H, −NH at pyrimidine ring), 6.83 (d, 2H, J = 7.26 Hz, Ar-H), 7.31 (d, 2H, J = 8.10 Hz, Ar-H), 7.49 (d, 2H, J = 7.49 Hz, Ar-H), 7.60 (t, 1H, Ar-H), 7.64 (t, 2H, Ar-H), 7.57 (d, 2H, J = 7.63 Hz, Ar-H at pyridine ring); m/z: 372.12 (100.0%), 373.13 (23.0%), 374.13 (3.1%), 373.12 (1.5%); ^{13}C NMR (300 MHz, $CDCl_3$) δ (ppm): 55.87 (−OCH_3), 136.9, 151.4 (C−O−C at pyran ring) 34.8 (−CH at pyran ring), 164.8 (C−NH_2), 160.9 (>C=O), 119.6, 128.0, 135.8, 137.2 (carbons at pyridine ring), 150.0 (−C=N at pyridine ring), 128.6, 131.2, 131.8, 132.9, 133.6, 134.9, 135.4, 136.2 (aromatic carbons); Anal. Calcd. for $C_{21}H_{16}N_4O_3$ (372.37): C, 67.73; H, 4.33; N, 15.05. Found: C, 67.70; H, 4.31; N, 15.01%.

4.6. General preparation of 4-amino-5-(4-methoxyphenyl)-1H-substitutedchromeno[2,3-d]pyrimidine-2(5H)-thione **8**(**a**–**e**)

A mixture of compound **4**(**a**–**e**) (2.0 mmol) and thiourea (152.0 mg, 2.0 mmol) with catalytic amount of sodium ethoxide in ethanol (15 ml) was refluxed on water bath for 6–7 h (Monitored by TLC). After completion of reaction, the reaction mixture was poured in crushed ice (50 g) and neutralized by the diluted hydrochloric acid (1:1). The separated product was collected by the filtration and washed with water. The crude product was purified by crystallization from absolute ethanol **8**(**a**–**e**).

4.6.1. 4-Amino-5-(4-methoxyphenyl)-1H-chromeno[2,3-d] pyrimidine-2(5H)-thione (**8a**)

185–187 °C (ethanol), Cream solid, Yield 65%, IR (KBr) ν (cm^{-1}): 2846 (−OCH_3), 3430 (−NH stretching at −NH_2), 3034, 1613 (−NH bending at −NH_2), 3331 (−NH stretching at pyrimidine ring), 1573 (−C=N pyrimidine ring), 1505 (−NH bending at pyrimidine ring), 1454, 1250 (C−O−C at pyran ring), 1180 (>C=S stretching); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 3.84 (−OCH_3), 4.74 (s, 1H, pyran −CH), 8.14 (s, 2H, −NH_2), 8.15 (s, 1H, −NH at pyrimidine ring), 7.11 (d, 2H, J = 7.25 Hz, Ar-H), 7.21 (d, 2H, J = 8.10 Hz, Ar-H), 7.37 (d, 2H, J = 7.41 Hz, Ar-H), 6.83–6.97 (t, 2H, Ar-H); m/z: 337.09 (100.0%), 338.09 (21.6%), 339.08 (4.5%), 339.10 (1.8%); ^{13}C NMR (300 MHz, $CDCl_3$) δ (ppm): 55.46 (−OCH_3), 153.3, 149.9 (C−O−C at pyran ring), 34.4 (−CH at pyran ring), 164.6 (C−NH_2), 55.8 (−OCH_3), 182.5 (>C=S), 121.5, 122.2, 123.4, 125.1, 125.6, 128.2, 128.8, 129.3, 131.0, 131.3 (aromatic carbons); Anal. Calcd. for $C_{18}H_{15}N_3O_2S$ (337.39): C, 64.08; H, 4.48; N, 12.45. Found: C, 64.10; H, 4.47; N, 12.41%.

4.6.2. 4-Amino-8-hydroxy-5-(4-methoxyphenyl)-1,5-dihydro-2H-chromeno[2,3-d]pyrimidine-2-thione (**8b**)

176–178 °C (ethanol), Orange solid, Yield 71%, (KBr) ν (cm^{-1}): 2855 (−OCH_3), 3438, (−NH stretching at −NH_2), 3030, 1610 (−NH bending at −NH_2), 3316 (−NH stretching at pyrimidine ring), 3210 (−OH), 1567 (−C=N pyrimidine ring), 1525 (−NH bending at pyrimidine ring), 1452, 1250 (C−O−C at pyran ring), 1179 (>C=S stretching); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 3.88 (−OCH_3), 4.80 (s, 1H, pyran −CH), 8.09 (s, 2H, −NH_2), 8.22 (s, 1H, −NH at pyrimidine ring), 9.05 (s, 1H, −OH), 7.10 (d, 2H, J = 7.56 Hz, Ar-H), 7.43 (d, 1H, J = 8.89 Hz, Ar-H), 7.73 (d, 2H, J = 7.33 Hz, Ar-H), 8.10 (d, 2H, J = 8.41 Hz, Ar-H); m/z: 353.08 (100.0%), 354.09 (19.8%), 355.08 (4.7%), 355.09 (2.6%), 354.08 (1.9%); ^{13}C NMR (300 MHz, $CDCl_3$) δ (ppm): 55.40 (−OCH_3), 154.5, 149.9 (C−O−C at pyran ring), 34.0 (−CH at pyran ring), 164.6 (C−NH_2), 180.4 (>C=S), 55.8 (OCH_3), 162.9 (C−OH), 100.4, 100.5, 112.2, 123.2, 123.5, 125.8, 126.0, 128.4, 129.2, 130.3, 131.5 (aromatic carbons); Anal. Calcd. for $C_{18}H_{15}N_3O_3S$ (353.39): C, 61.18; H, 4.28; N, 11.89. Found: C, 61.21; H, 4.30; N, 11.92%.

4.6.3. 8-Amino-7-(4-methoxyphenyl)-7,11-dihydro-10H-benzo[7,8] chromeno[2,3-d]pyrimidine-10-thione (**8c**)

178–179 °C (ethanol), Green solid, Yield 75%, (KBr) ν (cm^{-1}): 3430 (−NH stretching at −NH_2), 3315 (−NH stretching at pyrimidine ring), 3050, 1610 (−NH bending at −NH_2), 2840(−OCH_3), 1570 (−C=N pyrimidine ring), 1505 (−NH bending at pyrimidine ring), 1450, 1250 (C−O−C at pyran ring), 1185 (>C=S stretching); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 3.75 (−OCH_3), 4.80 (s, 1H, pyran −CH), 8.17 (s, 2H, −NH_2), 8.10 (s, 1H, −NH at pyrimidine ring), 6.90 (d, 2H, J = 7.12 Hz, Ar-H), 7.28 (d, 2H, J = 8.01 Hz, Ar-H), 7.36 (d, 2H, J = 7.48 Hz, Ar-H), 7.54 (d, 2H, J = 8.23 Hz, Ar-H), 8.10 (t, 2H, Ar-H); m/z: 387.10 (100.0%), 388.11 (24.1%), 389.10 (4.8%), 389.11 (3.4%), 388.10 (1.9%), 390.10 (1.1%); ^{13}C NMR (300 MHz, $CDCl_3$) δ (ppm): 55.42 (−OCH_3), 143.0, 149.2 (C−O−C at pyran ring), 34.8 (−CH at pyran ring), 165.6 (C−NH_2), 181.4 (>C=S), 119.2, 120.5, 123.5, 123.6, 123.2, 125.3, 125.9, 126.4, 126.6, 127.9, 128.4, 129.2, 130.3, 131.0 (aromatic carbons); Anal. Calcd. for $C_{22}H_{17}N_3O_2S$ (387.45): C, 68.20; H, 4.42; N, 10.85. Found: C, 68.17; H, 4.44; N, 10.84%.

4.6.4. 11-Amino-12-(4-methoxyphenyl)-8,12-dihydro-9H-benzo [5,6]chromeno[2,3-d]pyrimidine-9-thione (**8d**)

200–203 °C (ethanol), Green solid, Yield 88%, (KBr) ν (cm^{-1}): 3446 (−NH stretching at −NH_2), 3050, 1605, (−NH bending at −NH_2), 3301 (−NH stretching at pyrimidine ring), 2844 (−OCH_3), 1573 (−C=N pyrimidine ring), 1515 (−NH bending at pyrimidine ring), 1445, 1250 (C−O−C at pyran ring), 1180 (>C=S stretching); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 3.86 (−OCH_3), 4.80 (s, 1H, pyran −CH), 8.18 (s, 2H, −NH_2), 8.10 (s, 1H, −NH at pyrimidine ring), 6.60 (d, 2H, J = 7.29 Hz, Ar-H), 7.21 (d, 2H, J = 8.10 Hz, Ar-H), 7.51 (d, 2H, J = 7.57 Hz, Ar-H), 7.66 (d, 2H, J = 8.30 Hz, Ar-H), 7.90 (t, 2H, Ar-H); m/z: 387.10 (100.0%), 388.11 (24.1%), 389.10 (4.8%), 389.11 (3.4%), 388.10 (1.9%), 390.10 (1.1%); ^{13}C NMR (300 MHz, $CDCl_3$) δ (ppm): 55.41(−OCH_3), 149.0, 150.2 (C−O−C at pyran ring), 35.9 (−CH at pyran ring), 163.9 (C−NH_2), 180.4 (>C=S), 123.2, 122.7, 128.8, 129.6, 130.2, 131.8, 132.9, 133.4, 133.6, 134.9, 135.4, 135.2, 135.3, 136.0 (aromatic carbons); Anal. Calcd. for $C_{22}H_{17}N_3O_2S$ (387.45): C, 68.20; H, 4.42; N, 10.85. Found: C, 68.18; H, 4.43; N, 10.87%.

4.6.5. 8-Amino-7-(4-methoxyphenyl)-7,11-dihydro-10H-pyrimido [5′,4′:5,6]pyrano[3,2-h]quinoline-10-thione (**8e**)

190–192 °C (ethanol), Yellow solid, Yield 65%, (KBr) ν (cm^{-1}): 3438 (−NH stretching at −NH_2), 3329 (−NH stretching at pyrimidine ring), 3043, 2838(−OCH_3), 1612 (−NH bending at NH_2), 1576 (−C=N pyrimidine ring), 1506 (−NH bending at pyrimidine ring), 1454, 1260 (C−O−C at pyran ring), 1175 (>C=S Stretching); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 28.55(−OCH_3), 4.81 (s, 1H, pyran −CH), 8.10 (s, 2H, −NH_2), 8.12 (s, 1H, −NH at pyrimidine ring), 6.82 (d, 2H, J = 7.22 Hz, Ar-H), 7.25 (d, 2H, J = 8.11 Hz, Ar-H), 7.38 (d, 2H, J = 7.58 Hz, Ar-H), 7.60 (t, 1H, Ar-H), 7.97 (t, 2H, Ar-H), 7.47 (d, 2H, J = 7.6 Hz, Ar-H at pyridine ring); m/z: 342.11 (100.0%), 343.12 (21.9%), 344.12 (2.7%), 343.11 (1.5%); m/z: 388.10 (100.0%), 389.10 (25.1%), 390.10 (5.5%), 390.11 (2.5%), 391.10 (1.1%); ^{13}C NMR (300 MHz, $CDCl_3$) δ (ppm): 55.44 (−OCH_3),149.0–150.2 (C−O−C at pyran ring) 36.8 (−CH at pyran ring), 164.9 (C−NH_2), 180.7 (>C=S), 120.1, 122.2, 130.3, 134.5 (carbons at pyridine ring), 149.4 (−C=N at pyridine ring), 129.6, 130.2, 131.8, 132.9, 133.6, 134.9, 135.4, 136.2 (aromatic carbons); Anal. Calcd. for $C_{21}H_{16}N_4O_2S$ (388.44): C, 64.93; H, 4.15; N, 14.42. Found: C, 64.90; H, 4.13; N, 14.39%.

4.7. In vitro antibacterial and antifungal activity

A definition of the minimum inhibitory concentration MIC is “the lowest concentration which resulted in maintenance or reduction of inoculums viability”. The determination of the MIC involves a semi quantitative test procedure which gives an approximation to the

least concentration of antimicrobial agent needed to prevent microbial growth. The serial dilution technique [29] was applied for the determination of MIC of the tested compounds against four species of bacterial strains *S. aureus* [ATCC-25923] and *S. pyogenes* [MTCC-443] as Gram-positive, *E. coli* [ATCC-25922] and *P. aeruginosa* [MTCC-441] as Gram-negative and one species of fungal strain *A. niger* [MTCC-282]. Dilution series were set up with 62.5, 125, 250, 500 and 1000 μg/ml of nutrient broth medium to each tube, 1000 μL of standardized suspension of the test microbes (107 cell/ml) were added and incubated at 37 °C for 24 h.

4.8. In vitro antitubercular activity

The *in vitro* activity of the compounds against *M. tuberculosis* $H_{37}Rv$ was determined by agar micro dilution technique [30]. Twofold dilutions of each test compound were added to 7H10 agar and *M. tuberculosis* $H_{37}Rv$ was used as test organism. MIC is the concentration of the compound that completely inhibits the growth and colony forming ability of *M. tuberculosis*. In a 24 well plate 3 mL middle brook 7H10 agar medium with OADC supplement was dispensed in each well. The test compound was added to the middle brook medium agar before in duplicate so that the final concentration of the test compound in each well was 1000, 500, 250, 125, and 62.5 μg/mL, respectively. The known CFU of the $H_{37}Rv$ culture was dispensed on top of agar in each well in a negative pressure biosafety hood. The plates were then incubated at 37 °C in CO_2 incubator. The concentration at which complete inhibition of colonies was observed was taken as MIC of test drug.

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